747

A New Method for Measuring the Development of Rancidity[a,b,c]

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A rapid, objective and precise method has been devised for the determination of the relative stabilities of dry dog food materials containing 5% or more fat. The method is based upon the colorimetric determination of volatile carbonyl containing compounds formed when the fat becomes rancid.

In a study of the effect of various antioxidants in fat added to dry dog food it was found very difficult to evaluate rancidity by the usual organoleptic methods. The Schaal oven stability results obtained for the dry dog food with added fat used in this study lacked precision (Neumer and Dugan, *4*); this fact, together with the fact that the Schaal oven method lacks an objective criterion by which rancidity is ascertained, prompted the development of a rapid and precise method for determining the relative stabilities of "dry" food materials which contain 5% or more of fat.

An accelerated method for determining the relative stabilities of cereal type products has been reported by James *(1)*. The method was based upon the quantitative determination, by reduction of $KMnO_4$, of oxidizable materials in the gases exhausted from a cereal sample that was aerated at 97° C. It was concluded that the accelerated formation of those materials could be correlated with the onset of rancidity.

Since it is generally believed that compounds imparting the characteristic odor to rancid fat are primarily those containing functional carbonyl groups, it seemed plausible to expect that, at the time rancidity can be detected organoleptically in the gases exhausted from an aerated meal sample, an increased number of carbonyl compounds in the effluent gases could be detected by physico-chemical means.

A method by which the quantitative determination of traces of carbonyl compounds could be effected was reported by Lappin and Clark *(3)*. A rapid, objective, and precise method was thus developed for the determination of the relative stabilities of meals that contain fat by the adaptation of this method, and by modifying the standard A.O.M. apparatus *(2)* in such a manner that the relative concentrations of carbonyl compounds in the gases exhausted from a meal sample undergoing accelerated oxidation could be determined at periodic intervals.

To determine the applicability of this method for accelerated stability testing, a study was conducted on a progressively oxidized dry dog meal containing added fat. This study was made to compare the production of carbonyl compounds as measured by the newly devised accelerated stability test with the concentration of carbonyl compounds and peroxides in the meal-fat.

EXPERIMENTAL PROCEDURE

A. The apparatus and procedure used in the determination of relative stabilities by the "carbonyl" method are as follows: An individual unit of the modified A.O.M. apparatus *(2)* is shown in Figure 1. Features of the apparatus are:

(a) Calibrated capillaries (see i).
(b) Gum rubber connections.
(c) One-hole rubber stoppers.
(d) Standard A.O.M. oil bath.
(e) Rack designed to hold 18 U-tubes.
(f) Hydrogenated tallow.
(g) Pyrex U-tubes (20 x 200 mm.).
(h) Sample.
(i) Capillary tubing (½ mm.). Capillaries A and I, when attached in series to the air supply manifold, deliver 0.60 cc. of air/sec.
(j) Two-hole cork stopper.
(k) Test tube, 16 x 150 mm. calibrated to contain 10 ml.
(l) Benzene.

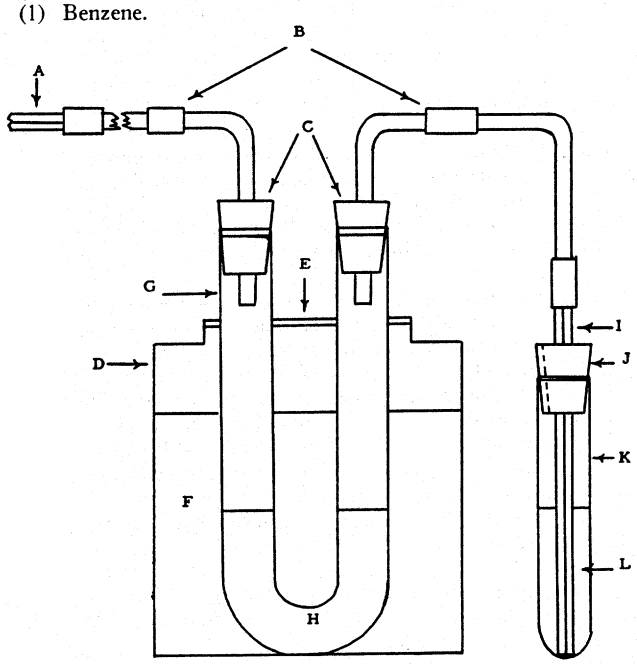

Figure 1. Apparatus used in the determination of the "carbonyl" stabilities of dog foods.

When the relative stability of a meal sample was to be determined, 20 g. of the material to be tested was placed in the U-tube and firmed by tapping so that the sample offered uniform resistance to air flow. After bringing the sample to the temperature of the bath (97.5° C.), the inlet tube was attached, the time recorded, and after allowing aeration to occur until excess water had been driven from the sample (generally 2 hours), the exit capillary was attached.

At periodic intervals, the lengths of which were determined by the stability of the sample and the approach of the break in the induction period, the effluent gases were allowed to pass through 10 ml. of reagent grade, thiophene-free benzene for a

[a] A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act. The contract is supervised by the Eastern Regional Research Laboratory, Bureau of Agricultural and Industrial Chemistry, USDA.

[b] Journal Paper Number 66, American Meat Institute Foundation.

[c] Presented at the 12th Annual Meeting of the Institute of Food Technologists, Grand Rapids, Michigan, June 8-18, 1952.

period of ½ hour; the time at which the carbonyl sample was collected was recorded. The relative concentration of carbonyl compounds in a 1-ml. aliquot of each 10 ml. effluent carbonyl sample was then determined according to the method of Lappin and Clark *(3)*. If their procedure is followed exactly, a volume of solution is obtained, the optical density of which can be measured at 480 mμ without further dilution by means of a Coleman Junior spectrophotometer.

B. The data used for comparing the production of carbonyl compounds by a rancid dog meal and the peroxide value of the meal fat were obtained in the following manner:

Sixteen 20-g. samples of a ration were prepared by mixing 148 g. of a Choice White grease (F.F.A. = 1.2%, O.P.V. = 2. A.O.M. stability = 8½ hrs.) with 1480 g. of dry dog food,[d] from which the fat had been previously extracted (Soxhlet) with 30-60° C. petroleum ether. These samples were placed in U-tubes and simultaneously aerated in the manner used for determining the "carbonyl" stability (see Experimental Procedure A).

At 2-hour intervals, starting with the 24th hour of aeration, an effluent carbonyl sample was collected from one of the sixteen samples, and the relative concentration of carbonyl compounds present determined by the method of Lappin and Clark.

Aeration of this meal sample was discontinued immediately after the collection of the effluent carbonyl sample while aeration of the remaining samples was continued until each in turn was removed from the bath. A desiccated 26 x 60 mm. fat-extraction thimble containing approximately 5.5 g. of the meal was weighed to the nearest mg. The sample was then continuously extracted with 35 ml. of thiophene free, reagent grade benzene for ½ hour in a Bailey-Walker apparatus. The thimble containing the extracted meal was dried in an 80-90° C. oven for 16 hours, reweighed, and the weight of fat obtained by difference.

The benzene-fat extract was diluted such that one one-thousandth of the quantity of extracted fat was present in 1 ml. of benzene solution. The concentration of carbonyl compounds in the diluted benzene-fat solution was determined using a modified procedure of Lappin and Clark. To 1.00 ml. of a benzene solution containing 0.5 to 0.8 mg. of fat/ml. in a 25 x 200 mm. test tube is added 1.00 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in carbonyl free methanol. The 2,4-dinitrophenylhydrazones are formed in the usual manner.[e] To the resulting solution of the 2,4-dinitrophenylhydrazones is added, with shaking, 5.5 ml. of a solution prepared in the following manner:

To 80 ml. of carbonyl free n-butanol is added a solution of 10 g. of KOH in 24 ml. water; the resulting solution is made homogeneous by adding 30 ml. of carbonyl free methanol.

The optical density (O.D.) of the resulting solution was read at 480 mμ with a Coleman Junior spectrophotometer, and the carbonyl concentration determined by comparing the O.D. of the fat carbonyl sample with a Beer's Law curve established by running an identical analysis on 10^{-4} to 10^{-6} molar solutions of vanillin in benzene. The concentration of carbonyl compounds in the fat was expressed as the carbonyl value, defined as the number of millimoles of functional carbonyl groups[f] present in 1 kg. of fat.

Evidence was obtained from reduced and non-reduced rancid fat samples which indicated that peroxides do not interfere with the above carbonyl determination.

An additional 8 to 10 g. sample of the oxidized meal was continuously extracted for 45 minutes with peroxide-free ethyl ether, and the weight of fat in the ether extract was determined in a manner similar to that used in the carbonyl value determination. The ether was vacuum distilled from the extract and the peroxide value of the meal-fat was then determined by the method of Wheeler *(5)*.

[d] Gaines Meal, purchased from the Gaines Dog Food Division, General Foods Corporation, Kankakee, Illinois.

[e] Five minute heating period at 100° C.

[f] A functional carbonyl group = any carbonyl group or immediate carbonyl precursor which will react with 2,4-dinitrophenylhydrazine under the conditions of this test to yield a 2,4-dinitrophenylhydrazone.

DISCUSSION

The stabilities of dry dog food samples containing 5% added Choice White grease fortified with 0.01% and 0.02% butylated hydroxyanisole (B.H.A.) were determined by the "carbonyl" method. When the relative concentrations of carbonyl compounds in the collected effluent samples, expressed in the units Io/I, are plotted against time of aeration, the curves which result, such as those in Figure 2, possess the characteristic features of induction curves.

It has been found desirable to define the relative stability of a feed as the time of aeration required for a developed, effluent carbonyl sample to attain a specific value of Io/I. This value of Io/I, the index of rancidity, is so selected that in each case under consideration, the line defined by the index of rancidity and the induction curve intersect beyond the break in the induction period, and on that portion of the curve whose slope is sufficiently great and well defined. The carbonyl stability results in our studies were determined using Io/I = 3 as the index of rancidity.

The relative stabilities of eight replicate dog food samples containing added fat were determined using the above criterion; the average deviation from the mean stability (50 hours) was 1%.

The study conducted to compare the production of carbonyl compounds by a rancid meal with the peroxide value of the meal-fat was designed to determine the following relationships:

(a) The relationship between the rate of production of peroxides and the rate of production of carbonyl compounds in the fat present in the ration undergoing accelerated oxidation.

(b) The relationship between the peroxide value and carbonyl value of the meal-fat, and the optical densities

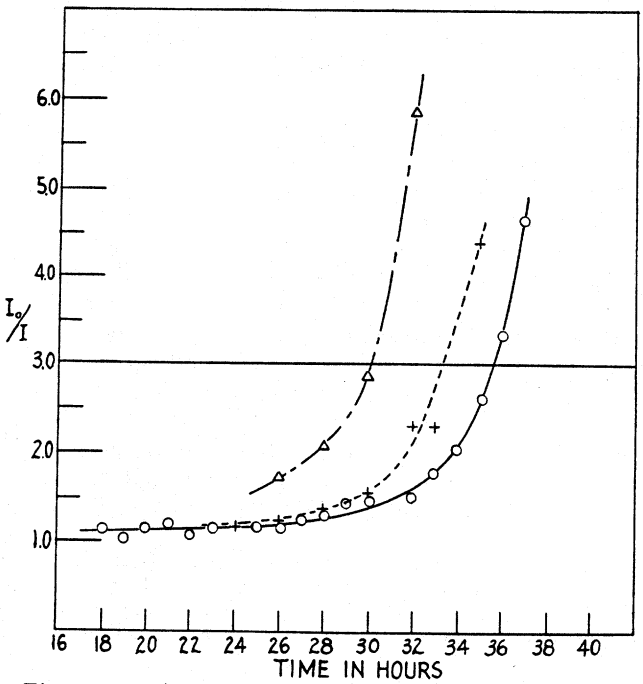

Figure 2. Io/I (480 mμ). For developed effluent carbonyl sample versus time of aeration. △, control; +, 0.01% BHA; ○, 0.02% BHA. (Dry dog food with 5% added Choice White grease.)

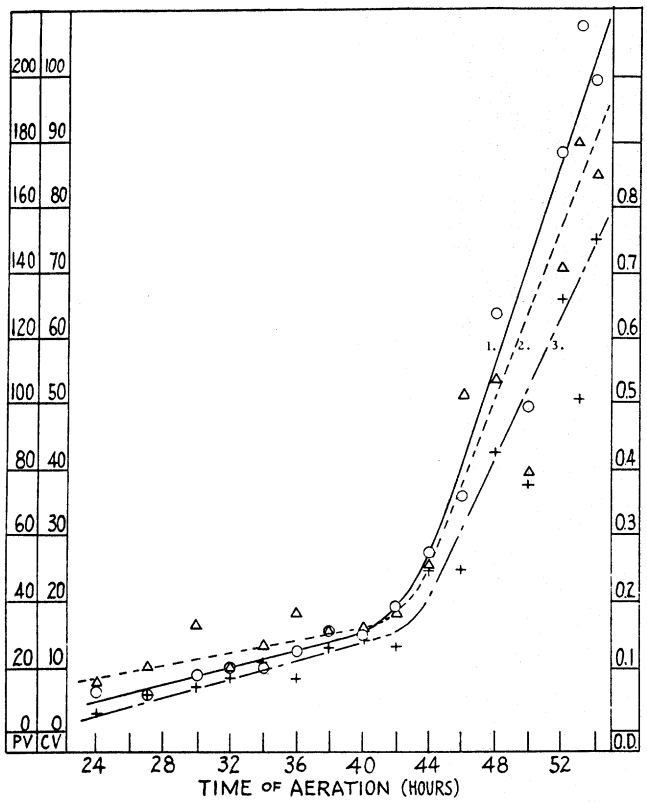

Figure 3. The changes occurring in the meal-fat P.V. (○-○) and C.V. (△—△), and the O.D. (480 mμ.) of the developed effluent carbonyl sample (+—·—+) with increasing time of aeration. Time for the P.V. and C.V. curves is that indicated on the abscissa plus ½ hr., for the O.D. curve, plus ¼ hr.

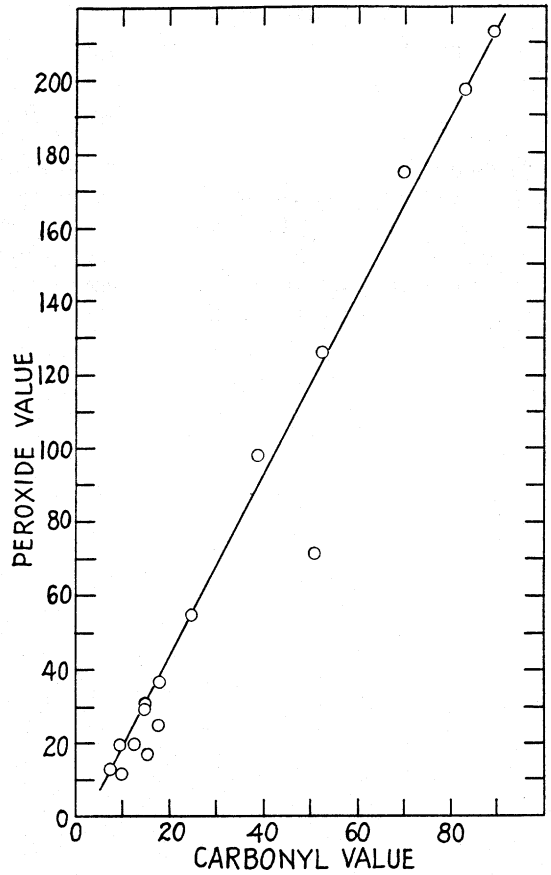

Figure 4. The peroxide value versus carbonyl value of meal-fat extracted from meal aerated for varying periods of time.

of developed effluent carbonyl samples, at any time during aeration.

The plots of the data obtained in the latter investigation, the peroxide value (P.V.), carbonyl value (C.V.), and the optical density (O.D.) of the effluent carbonyl sample, *versus* time of aeration are shown in Figure 3.

It can be seen from curves 1 and 2 of Figure 3, that a rapidly accelerated production of carbonyl compounds in the fat in the meal undergoing accelerated oxidation occurred at exactly the same time as did that of the peroxides; i.e., the break in the P.V. and C.V. *versus* time curves occurred simultaneously. Moreover, the break in the O.D. of effluent carbonyl sample *versus* time curve occurred only 0.75 hour later than did the breaks in curves 1 and 2.

When the P.V. of the meal-fat at any time of aeration was plotted against the C.V. at the same time, the linear curve represented in Figure 4 resulted. Thus, over the time interval studied, it was determined that mM. of meal-fat peroxides = 1.2 mM. of functional carbonyl groups in the meal-fat.

The linear curve (Figure 5) which resulted when the peroxide value of the meal-fat was plotted against the O.D. of the effluent carbonyl sample indicated the soundness of the criterion used in determining the relative stabilities of meal samples by the "carbonyl" method.

It is believed that the "carbonyl" method affords a rapid, precise and objective means for comparing the stabilities of dry foods containing 5% or more fat.

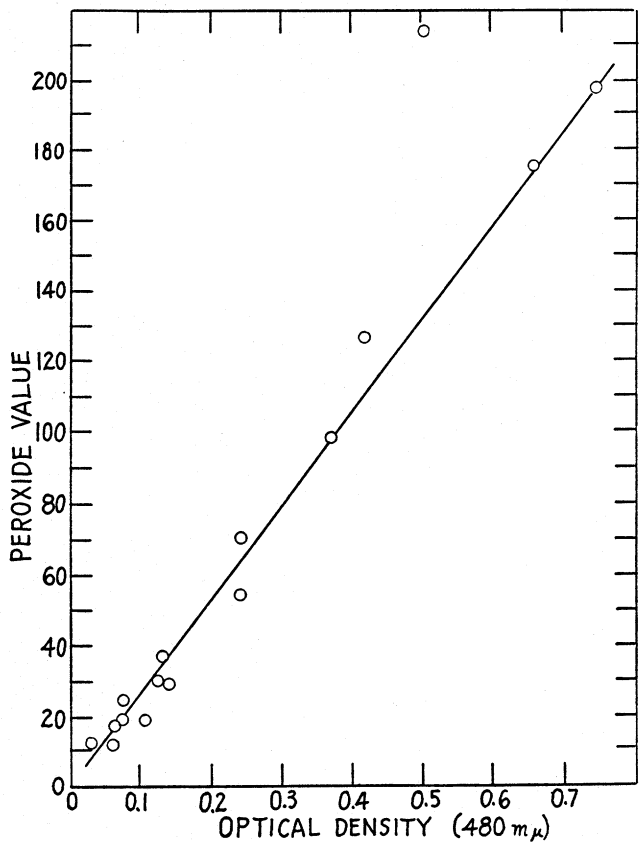

Figure 5. Relationship between the peroxide value of the meal-fat and the O.D. of a developed effluent carbonyl sample.

SUMMARY

A method has been developed for determining the relative stabilities of dry dog food materials containing fat. The method is a physico-chemical method based on the colorimetric determination of volatile "carbonyl" containing compounds formed during accelerated aging by air at elevated temperatures of food materials containing fat. Excellent correlation was obtained between the criterion for relative stability used in the "carbonyl" method and the peroxide value of the fat present in the meal undergoing accelerated oxidation.

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